

IN THE SPECIFICATION:

On page 31, line 16 through page 32, line 4, please replace with the following paragraph:

The CX/GFP/Puro construct demonstrates that transgenes of at least 4.5kb can be inserted into chimeric chickens. Using the cES cells described herein, chicken ES cells can be transfected with different or larger constructs. A very large transgene encoding part of the unrearranged human heavy chain immunoglobulin locus has been transfected into chicken ES cells. A 139 kilobase bacterial artificial chromosome (BAC) clone was co-transfected with the pCX-EGFP-CX-puro selectable marker into cES cells by co-lipofection of circular BAC DNA and linear selectable marker DNA. The BAC clone contains a human genomic DNA insert from an unrearranged immunoglobulin heavy chain locus and contains the most 3' variable region ( $V_H6-1$ ), all the diversity (D) segments, all the joining (J) segments, the Cmu (C $\mu$ ) and Cgamma (C $\gamma$ ) constant regions, the J-intronic enhancer, and all the intervening DNA between these elements. It also includes the human gene KIAA0125, a gene that encodes a non-translated RNA of unknown function that is found between  $V_H6-1$  and the D segment region. pCX-EGFP-CX-puro is a plasmid that contains the Enhanced Green Fluorescent Protein (EGFP) gene driven by the CX promoter (consisting of a cytomegalovirus enhancer and the chicken  $\beta$ -actin promoter) and a puromycin resistance gene driven by the same promoter. The cES cells transfected with this plasmid are green fluorescent and resistant to the antibiotic puromycin. The presence of the unrearranged human heavy chain locus in the transfected ES cells that were growing in the presence of puromycin was examined by PCR analysis of transgenes spread throughout the 139 kb construct. The primer sequences used in the PCR analysis were:

$V_H6-1$ :  
V6-1F      AGTGTCAGGGAGATGCCGTAT SEQ ID NO. 1  
TCA

V6-1R      ACTTCCCCTCACTGTGTCTCTT SEQ ID NO. 2  
G

D1-26:  
D1-26F      GGGCGCCTGGGTGGATTCTG SEQ ID NO. 3  
A

D1-26R      GTGGCCCCTAAACCTGAGTCT SEQ ID NO. 4  
GCT

D1-20:  
D1-20F      CCCGAGCACCGTCCCCATTGA SEQ ID NO. 5  
D1-20R      GTGCCGGTGATCCCTGTCTTT SEQ ID NO. 6  
CTG

□□:  
Mu1F      GCGGGAGTCGGCCACCATCAC SEQ ID NO. 7  
G

Mu1R      AGCACAGCCGCGCCCCAGTA SEQ ID NO. 8  
G

□□□  
Delta1F      TGGGGAGAGGAGAGCACAGT SEQ ID NO. 9  
Delta1R      GGCGGGCGTAGGGGTCAGC SEQ ID NO. 10

On page 35, lines 6-11, please replace with the following paragraph:

A 2687 bp fragment of chicken IgH switch and constant region (base 11-2697, Genebank #AB029075) was amplified from chicken genomic DNA. Primers Cu-1 (with BamH I site underlined) and Cu-2 (with EcoR I site underlined) were designed based on the above referenced sequence.

Cu-1: 5'-CTCGGATCCCAACAAACGGCACTCGATAATT-3' (SEQ ID NO. 11)

Cu-2: 5'-CTCGAATTCTTTCATTGACCTTCATTAACCGC-3' (SEQ ID NO. 12)

On page 36, lines 4-9, please replace with the following paragraph:

In another embodiment, a 2396 bp fragment of chicken IgL V region (base 24-2419, Genebank #M24403) was amplified from chicken genomic DNA. Primers CiGL5A (with Hpa I

sequence underlined) and CiG15B (with Hpa I sequence underlined) were designed based on the above-referenced sequence.

cIgL5A: 5'-CTCGTAAACGATGTTGTACTGAGGGATGTGG-3' (SEQ ID NO. 13)

cIgL5B: 5'-CTCGTAAACCGGTGAACAAGGATGTTTCAGTA-3' (SEQ ID NO. 14)

On page 42, line 4 through page 43, line 4, please replace with the following paragraph:

For example, RP11-329I4 contains VH6-1, VH3-7 and VH6-4, which can be identified with the following primer pairs:

Primer pair for VH6-1:

V6-1F      AGTGTCAGGGAGATGCCGTAT (SEQ ID NO. 1)  
TCA

V6-1R      ACTTCCCCTCACTGTGTCTCTT (SEQ ID NO. 2)  
G

Primer pair for VH3-7:

Vh3-7F      GGCTGAGCTGGGTTTTCTTG (SEQ ID NO. 15)  
TT

Vh3-7R      CTGTCGCCCCCTGGTGGTC (SEQ ID NO. 16)

Primer pair for VH4-4:

Vh4-4F      CCTGCACAAGAACATGAAACA (SEQ ID NO. 17)  
CCT

Vh4-4R      GACCCGGCCTCTTGCTCTG (SEQ ID NO. 18)

The other BAC used in conjunction with the V region BAC contains a majority of the D segments, all the J segments, the J-mu enhancer, and the constant regions mu, delta, gamma3 and gamma1.

This BAC (RP11-417P24) spans the D-gamma1 region in germline configuration and thus contains a wild type complete delta-gamma3 interval. This BAC has been sequenced in the course of the Human Genome Project and can be identified using the following sets of PCR primer pairs:

Amplifies a region near D1-26:

D1-26F      GGGCGCCTGGGTGGATTCTG    (SEQ ID NO. 3)

A

D1-26R      GTGGCCCCTAAACCTGAGTCT    (SEQ ID NO. 4)

GCT

Amplifies a region near D1-20:

D1-20F      CCCGAGCACCGTCCCCATTGA    (SEQ ID NO. 5)

D1-20R      GTGCCGGTGATCCCTGTCTTT    (SEQ ID NO. 6)

CTG

Amplifies part of the ~~C<sub>mu</sub>~~ C<sub>mu</sub> constant region:

Mu1F        GCGGGAGTCGGCCACCATCAC    (SEQ ID NO. 7)

G

Mu1R        AGCACAGCCGCCGCCCCAGTA    (SEQ ID NO. 8)

G

Amplifies part of the ~~C<sub>delta</sub>~~ C<sub>delta</sub> constant region:

Delta1F      TGGGGAGAGGAGAGCACAGT    (SEQ ID NO. 9)

Delta1R      GGCGGGCGTAGGGGTCAGC    (SEQ ID NO. 10)

Amplifies a part of the delta-gamma3 interval:

UncloF       GCTGTTGGCCTTTATTTTCTAT    (SEQ ID NO. 19)

TG

UncloR      ATTTGCACCATTTTCCTGAGTTG (SEQ ID NO. 20)

Amplifies another part of the delta-gamma3 interval:

Unclo2F      GTGGGTGATAGAATTTGGTGT (SEQ ID NO. 21)

TTG

Unclo2R      GTGGTGGGCAGGATGGGATG (SEQ ID NO. 22)

AT

A single BAC, such as CTD-2005N2, spanning the region from the 3' most V, VH6-1, through the delta constant region, can also be transfected into cES cells for the purpose of expression of a primary repertoire consisting of IgM and IgD only.

On page 43, lines 10-19, please replace with the following paragraph:

For the kappa light chain, a single BAC containing several VK genes, all 5 Jk's, Ckappa constant region and enhancers is transfected into ES cells. Such BACs are RP11-601N4 (with 2 variable region genes present, VK5-2 and VK4-1), RP11-1134E24 (with 4 VK's, VK1-6, VK1-5, VK5-2, and VK4-1), and RP11-15J7 (with the same complement of kappa light chain elements as RP11-1134E24). These BACs can be identified with by PCR with the following sets of PCR primer pairs:

Amplifies part of the Jk region:

JkappaF      ATGCCAGGGACTCTAACAAAC (SEQ ID NO. 23)

TTC

JkappaR      TTCCCCTCAACAAAAACCTCTC (SEQ ID NO. 24)

Amplifies a part of the Ck constant region:

CkappaF      AGCTCGCCCGTCACAAAGA (SEQ ID NO. 25)

CkappaR AGGGGAAAACAAGGAAGCAA (SEQ ID NO. 26)

GTC

Amplifies part of the Vk4-1 variable gene:

Vk4-1F GAGAGGGCCACCATCAACTG (SEQ ID NO. 27)

Vk4-1R AACCTCCAACGAATAAATCAA (SEQ ID NO. 28)

GA

Amplifies part of the Vk5-2 variable gene:

Vk5-2F AAGTCCCCTGCATATCCACAAA (SEQ ID NO. 29)

A

Vk5-2R GCTGAGGCAATCCCACTGAGA (SEQ ID NO. 30)